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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 09:25:21 ON 20 SEP 2004

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 09:25:36 ON 20 SEP 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s alpha amylase#
FILE 'MEDLINE'
        487067 ALPHA
         21005 AMYLASE#
L1        4696 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'SCISEARCH'
        693102 ALPHA
         17088 AMYLASE#
L2        7691 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'LIFESCI'
        156463 "ALPHA"
          4444 AMYLASE#
L3        2675 ALPHA AMYLASE#
                 ("ALPHA"(W)AMYLASE#)

FILE 'BIOTECHDS'
         26670 ALPHA
          5371 AMYLASE#
L4        3314 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'BIOSIS'
        630505 ALPHA
         27737 AMYLASE#
L5        9922 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'EMBASE'
        548887 "ALPHA"
         15457 AMYLASE#
L6        3452 ALPHA AMYLASE#
                 ("ALPHA"(W)AMYLASE#)

FILE 'HCAPLUS'
       1497037 ALPHA
         44165 AMYLASE#
L7       18408 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'NTIS'
         28638 ALPHA
           164 AMYLASE#
L8          60 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)
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FILE 'ESBIOBASE'
        199842 ALPHA
          4183 AMYLASE#
L9        2006 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'BIOTECHNO'
        189431 ALPHA
          4194 AMYLASE#
L10       2130 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'WPIDS'
        177480 ALPHA
          5609 AMYLASE#
L11       2332 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

TOTAL FOR ALL FILES
L12      56686 ALPHA AMYLASE#

=> s l12(5a)bacillus
FILE 'MEDLINE'
         47589 BACILLUS
L13        485 L1 (5A)BACILLUS

FILE 'SCISEARCH'
         46835 BACILLUS
L14        711 L2 (5A)BACILLUS

FILE 'LIFESCI'
         24790 BACILLUS
L15        497 L3 (5A)BACILLUS

FILE 'BIOTECHDS'
         16387 BACILLUS
L16        998 L4 (5A)BACILLUS

FILE 'BIOSIS'
         65930 BACILLUS
L17       1017 L5 (5A)BACILLUS

FILE 'EMBASE'
         34393 BACILLUS
L18        475 L6 (5A)BACILLUS

FILE 'HCAPLUS'
         81331 BACILLUS
L19       2138 L7 (5A)BACILLUS

FILE 'NTIS'
          1650 BACILLUS
L20          5 L8 (5A)BACILLUS

FILE 'ESBIOBASE'
         14284 BACILLUS
L21        241 L9 (5A)BACILLUS

FILE 'BIOTECHNO'
         19958 BACILLUS
L22        415 L10(5A)BACILLUS

FILE 'WPIDS'
         12118 BACILLUS
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L23         206 L11(5A)BACILLUS

TOTAL FOR ALL FILES
L24        7188 L12(5A) BACILLUS

=> s l12(5a)(muta? or variant#)
FILE 'MEDLINE'
        453853 MUTA?
         98656 VARIANT#
L25         105 L1 (5A)(MUTA? OR VARIANT#)

FILE 'SCISEARCH'
        434042 MUTA?
        106002 VARIANT#
L26         130 L2 (5A)(MUTA? OR VARIANT#)

FILE 'LIFESCI'
        204046 MUTA?
         33761 VARIANT#
L27          93 L3 (5A)(MUTA? OR VARIANT#)

FILE 'BIOTECHDS'
         39136 MUTA?
         12875 VARIANT#
L28         166 L4 (5A)(MUTA? OR VARIANT#)

FILE 'BIOSIS'
        500804 MUTA?
        102443 VARIANT#
L29         223 L5 (5A)(MUTA? OR VARIANT#)

FILE 'EMBASE'
        375941 MUTA?
         85981 VARIANT#
L30          96 L6 (5A)(MUTA? OR VARIANT#)

FILE 'HCAPLUS'
        463722 MUTA?
         96747 VARIANT#
L31         383 L7 (5A)(MUTA? OR VARIANT#)

FILE 'NTIS'
          9697 MUTA?
          4500 VARIANT#
L32          1 L8 (5A)(MUTA? OR VARIANT#)

FILE 'ESBIOBASE'
        222627 MUTA?
         38570 VARIANT#
L33         66 L9 (5A)(MUTA? OR VARIANT#)

FILE 'BIOTECHNO'
        242571 MUTA?
         41198 VARIANT#
L34         77 L10(5A)(MUTA? OR VARIANT#)

FILE 'WPIDS'
         25470 MUTA?
         24277 VARIANT#
L35         67 L11(5A)(MUTA? OR VARIANT#)

TOTAL FOR ALL FILES
L36        1407 L12(5A)(MUTA? OR VARIANT#)
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=> s l24 and l36
FILE 'MEDLINE'
L37          33 L13 AND L25

FILE 'SCISEARCH'
L38          38 L14 AND L26

FILE 'LIFESCI'
L39          32 L15 AND L27

FILE 'BIOTECHDS'
L40          88 L16 AND L28

FILE 'BIOSIS'
L41          54 L17 AND L29

FILE 'EMBASE'
L42          34 L18 AND L30

FILE 'HCAPLUS'
L43         163 L19 AND L31

FILE 'NTIS'
L44           0 L20 AND L32

FILE 'ESBIOBASE'
L45          11 L21 AND L33

FILE 'BIOTECHNO'
L46          22 L22 AND L34

FILE 'WPIDS'
L47          32 L23 AND L35

TOTAL FOR ALL FILES
L48         507 L24 AND L36

=> s l48 not 1997-2004/py
FILE 'MEDLINE'
       3836369 1997-2004/PY
L49          27 L37 NOT 1997-2004/PY

FILE 'SCISEARCH'
       7562964 1997-2004/PY
L50          30 L38 NOT 1997-2004/PY

FILE 'LIFESCI'
        821451 1997-2004/PY
L51          27 L39 NOT 1997-2004/PY

FILE 'BIOTECHDS'
        135411 1997-2004/PY
L52          58 L40 NOT 1997-2004/PY

FILE 'BIOSIS'
       4157553 1997-2004/PY
L53          44 L41 NOT 1997-2004/PY

FILE 'EMBASE'
       3427474 1997-2004/PY
L54          27 L42 NOT 1997-2004/PY

FILE 'HCAPLUS'
       6997733 1997-2004/PY
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L55          96 L43 NOT 1997-2004/PY

FILE 'NTIS'
        161239 1997-2004/PY
L56           0 L44 NOT 1997-2004/PY

FILE 'ESBIOBASE'
       2186826 1997-2004/PY
L57           4 L45 NOT 1997-2004/PY

FILE 'BIOTECHNO'
        829801 1997-2004/PY
L58          16 L46 NOT 1997-2004/PY

FILE 'WPIDS'
       5928527 1997-2004/PY
L59           5 L47 NOT 1997-2004/PY

TOTAL FOR ALL FILES
L60         334 L48 NOT 1997-2004/PY

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COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               24.06      24.27

STN INTERNATIONAL LOGOFF AT 09:29:40 ON 20 SEP 2004
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Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	707	(alpha adj amylase$1) same bacillus same (mutant$1 or variant$1)	US-PGPUB; USPAT	OR	OFF	2004/09/20 09:13
L2	1435	(mutant$1 or variant$1) near5 (stability or thermostabilty or calcium adj depend$8)	US-PGPUB; USPAT	OR	OFF	2004/09/20 09:15
L3	102	1 and 2	US-PGPUB; USPAT	OR	OFF	2004/09/20 09:16

PGPUB-DOCUMENT-NUMBER: 20040115779

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040115779 A1

TITLE: Fermentation process

PUBLICATION-DATE: June 17, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Olsen, Hans Sejr	Holte	NC	DK	
Pedersen, Sven	Gentofte	NC	DK	
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APPL-NO: 10/ 472256

DATE FILED: September 19, 2003

PCT-DATA:
APPL-NO: PCT/DK02/00179
DATE-FILED: Mar 19, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/105, 435/252.31 , 435/254.3

ABSTRACT:

The present invention relates to an improved process for producing a fermentation product.

---------- KWIC ---------

Detail Description Paragraph - DETX (85):
[0090] Other contemplated Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Furthermore Clark Ford presented a paper on Oct. 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct. 12-17, 1997, Abstract number: Abstract book p. 0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an Aspergillus awamori glucoamylase to improve the thermal stability. Other glucoamylases include Talaromyces glucoamylases, in particular derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus, Talaromyces duponti (U.S. Pat. No. 32,153), Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

Detail Description Paragraph - DETX (107):

[0112] The liquefaction step may be performed in the presence of an alpha-amylase derived from a microorganism or a plant. Preferred alpha-amylases are of fungal or bacterial origin. Bacillus alpha-amylases (often referred to as "Termamyl-like alpha-amylases"), variant and hybrids thereof, are specifically contemplated according to the invention. Well-known Termamyl-like alpha-amylases include alpha-amylase derived from a strain of B. licheniformis (commercially available as Termamyl.TM.), B. amyloliquefaciens, and B. stearothermophilus alpha-amylase (BSG). Other Termamyl-like alpha-amylases include alpha-amylase derived from a strain of the Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. In the context of the present invention a Termamyl-like alpha-amylase is an alpha-amylase as defined in WO 99/19467 on page 3, line 18 to page 6, line 27. Contemplated variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Contemplated alpha-amylase derived from a strain of Aspergillus includes Aspergillus oryzae and Aspergillus niger--amylases.

PGPUB-DOCUMENT-NUMBER: 20040096952

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040096952 A1

TITLE: Alpha-amylase variant with altered properties

PUBLICATION-DATE: May 20, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Svendsen, Allan	Horsholm		DK	
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APPL-NO: 10/ 477725

DATE FILED: November 14, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2001 00760	2001DK-PA 2001 00760	May 15, 2001
DK	PA 2001 00981	2001DK-PA 2001 00981	June 22, 2001
DK	PA 2001 00982	2001DK-PA 2001 00982	June 22, 2001
DK	PA 2001 00998	2001DK-PA 2001 00998	June 26, 2001
DK	PA 2001 00999	2001DK-PA 2001 00999	June 26, 2001
DK	PA 2001 01443	2001DK-PA 2001 01443	October 2, 2001

PCT-DATA:
APPL-NO: PCT/DK02/00319
DATE-FILED: May 15, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/202, 435/252.31 , 510/226 , 510/320

ABSTRACT:

The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits altered properties relative to the parent alpha-amylase.

---------- KWIC ---------

Detail Description Paragraph - DETX (622):
[0670] Variants of the invention may have altered oxidation stability, in particular higher oxidation stability, in comparison to the parent alpha-amylase. Increased oxidation stability is advantageous in, e.g., detergent compositions and descresed oxidation stability may be advantageous in composition for starch liquefaction. Oxidation stability may be determined as described in the "Material & Methods" section below.

Detail Description Paragraph - DETX (1373):
[1421] In an aspect, the invention relates to providing alpha-amylase variants with reduced sensitivity (or improved stability against denaturation) to anionic surfactants (in particular linear alkyl sulphonates (LAS)). These variants are provided by substituting, deleting or inserting an amino acid residue in the parent alpha-amylase as indicated below with a more hydrophilic amino acid residue. Such variants may be prepared by:

Detail Description Paragraph - DETX (1378):
[1426] Variants of the invention with reduced sensitivity to anionic surfactants, in particular linear alkyl sulphonates (LAS), include (using the Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8 numbering):

Detail Description Paragraph - DETX (1722):
[1770] In an aspect the invention relates to Termamyl-like alpha-amylase variant with increased stability at acidic pH and/or at high temperature in comparison to the parent alpha-amylase. Such variants are especially suitable for starch liquefaction.

Detail Description Paragraph - DETX (2155):
[2203] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (2233):
[2281] Amylases: Suitable amylases (alpha and/or beta) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from Bacillus, e.g., a special strain of B. licheniformis, described in more detail in GB 1,296,839. Examples of useful alpha-amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

Detail Description Paragraph - DETX (2291):
[2339] The below assays can be used to screening of Termamyl-like alpha-amylase variants having altered stability at high or low pH and/or under Ca.sup.2+ depleted conditions compared to the parent enzyme and Termamyl-like alpha-amylase.

Detail Description Paragraph - DETX (2303):
[2351] Stability Assay of Unpurified Variants

Detail Description Paragraph - DETX (2304):

[2352] Bacillus cultures expressing the variants to be analysed are grown for 21 hours at 37.degree. C. in 10 ml LB+chloramphenicol. 800 micro liter culture is mixed with 200 micro l citrate buffer, pH 4.5. A number of 70 micro l aliquots corresponding to the number of sample time points are made in PCR tubes and incubated at 70.degree. C. or 90.degree. C. for various time points (typically 5, 10, 15, 20, 25 and 30 minutes) in a PCR machine. The 0 min sample is not incubated at high temperature. Activity in the sample is measured by transferring 20 micro l to 200 micro l of the alpha-amylase PNP-G.sub.7 substrate MPR3 ((Boehringer Mannheim Cat. no. 1660730) as described below under "Assays for Alpha-Amylase Activity". Results are plotted as percentage activity (relative to the 0 time point) versus time, or stated as percentage residual activity after incubation for a certain period of time.

Detail Description Paragraph - DETX (2310):
[2358] Stability Determination of Purified Variants

Detail Description Paragraph - DETX (2311):
[2359] All stability trials of purified variants are made using the same set up. The method is as follows:

Detail Description Paragraph - DETX (2360):
[2406] The below listed variants are constructed as described in EXAMPLE 1 of WO 00/29560 (from Novozymes A/S) in the parent Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8.

Detail Description Paragraph - DETX (2790):
[2835] The below listed variants are constructed as described in EXAMPLE 1 of WO 00/37626 (from Novozymes A/S) in the parent Bacillus licheniformis alpha-amylase shown in SEQ ID NO: 8. The alterations of the variants are, as specified in the list below, insertion of an amino acid downstream of the amino acid which occupies the position, or deletion of the amino acid which occupies the position.

Claims Text - CLTX (1):
1. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions selected from the group of: 5, 6, 36, 37, 38, 39, 42, 45, 47, 63, 66, 69, 70, 71, 72, 74, 75, 76, 79, 82, 83, 86, 87, 89, 93, 112, 113, 117, 120, 137, 213, 216, 220, 223, 225, 226, 227, 229, 243, 245, 279, 282, 311, 321, 324, 352, 353, 354, 357, 361, 362, 364, 368, 390, 395, 397, 399, 400, 401, 425, 451, 452, 453, 466, 468, 470, 471, 478, wherein (a) the alteration(s) are independently (i) an insertion of an amino acid downstream of the amino acid which occupies the position, (ii) a deletion of the amino acid which occupies the position, or (iii) a substitution of the amino acid which occupies the position with a different amino acid, (b) the variant has alpha-amylase activity and (c) each position corresponds to a position of the amino acid sequence of the parent termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (bacillus licheniformis alph-amylase).

Claims Text - CLTX (2):
2. A variant of a parent Termamyl-like alpha-amylase, comprising one or more of the following substitutions. X1A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,-W,Y; X2R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V; X3A,R,N,D,C,Q,E,G,H,I,L,K,M,F,-P,S,T,W,Y; X4A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X13R,N,D,C,Q,E,G,H,K,M,P,S,T,W; X14A,R,D,C,G,K,M,P,W; X16R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V; X17A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,-S,T,W,Y,V; X18A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X20A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X23A,R,N,D,C,Q,E,G,H,I,L,M,F,-P,S,W,Y,V; X24A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X26A,D,C,E,G,H,I,L,M,F,P,S,T,W,V; X34A,R,N,C,Q,E,G,H,I,L,K,M,F,P,T,W,Y,V;

X35A,R,N,D,C,Q,E,G,H,K,M,F,P,S,T,W,Y,V; X49A,C,G,H,P,T;
X50A,R,N,C,Q,E,G,H,K,M,F,P,S,W; X51A,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V;
X52A,R,D,C,Q,E,G,H,K,P; X53A,D,C,G,H,K,M,P; X61A,R,N,D,C,Q,E,G,H,I,L,K,M,-
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X68A,R,D,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X73A,R,N,D,C,Q,E,G,H,K,M,P,S,T,W,-
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X91A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X92A,R,N,D,C,Q,E,G,H,I,L,M,F,-
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X106A,N,D,C,Q,E,G,H,I,L,K,M,P,S,T,Y,V; X108R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,-
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X127A,R,N,D,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X128A,R,N,D,C,Q,G,H,I,L,K,M,F,P,-
S,W,Y,V; X129A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V;
X130A,R,N,D,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V; X131A,R,N,D,C,Q,G,H,I,L,K,M,F,P,-
S,T,W,Y,V; X132R,N,D,C,Q,E,G,H,I,L,K,M,F,S,W,Y; X133R,N,D,C,M,T,W,V;
X134A,N,D,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X136A,R,N,D,C,E,G,H,I,L,K,M,F,P,-
S,T,W,Y,V; X138R,N,D,Q,E,G,I,K,M,P,S,T,W,V; X145A,R,N,D,C,Q,E,G,H,I,L,K,M,-
P,S,T,Y,V; X147A,R,N,D,C,Q,E,G,H,I,L,K,M,P,S,T,W,Y,V;
X148A,R,D,C,Q,E,G,H,I,L,K,M,F,P,T,W,Y,V; X149A,R,N,D,C,Q,E,G,H,L,K,M,F,P,-
S,T,W,Y,V; X150A,D,C,G,M,P,W,Y; X152A,R,N,C,Q,E,G,H,I,L,K,M,F,P,T,W,Y,V;
X153A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X154A,R,N,D,C,Q,E,G,H,I,L,K,-
M,F,P,S,T,W,Y,V; X155A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X156A,C,Q,E,G,I,L,M,F,P,S,T,W,V; X157R,I,L,M,F,P,S,T,W,Y,V; X158R,M,P,W,Y;
X164R,I,L,M,F,P,S,T,W,Y,V; X165A,N,D,C,Q,E,H,I,L,K,M,F,P,S- ,T,W,Y,V;
X167A,R,N,D,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X168A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,V; X169A,R,N,D,C,Q,E,G,H,M,P,S,W,Y,- V;
X170A,R,N,D,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X171A,R,N,D,C,Q,E,G,H,K,M,P,-
S,T,W,Y,V; X172A,N,D,C,Q,E,G,I,L,M,F,P,T,W,Y,V; X173A,N,D,C,Q,E,G,H,M,P,S,-
W,Y,V; X176A,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X179D,C,Q,E,H,I,L,K,M,F,-
P,W,Y,V; X180A,G,I,L,M,F,P,W,Y,V; X181G; X182A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P-
,S,T,W,Y,V; X184I,L,M,F,P,W,Y,V; X185R,I,L,M,F,P,S,T,W,Y,V;
X188A,R,N,Q,G,H,L,M,F,W,V; X189A,R,N,G,H,I,L,M,F,P,S,T,W,Y,V; X190N;
X191A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X193A,R,G,M,P,W,Y;
X196A,N,Q,G,H,I,L,M,P,S,T,W,V; X198A,R,G,M,P,W; X204R,L,M,F,P,T,W,Y,V;
X205A,G,H,I,L,M,F,P,W,Y,V; X206R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X209R,P,S,W,Y; X210A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,W,Y,V; X211E;
X214A,D,C,Q,E,G,I,L,M,F,P,S,T,Y,V; X217A,R,N,D,C,Q,G,H,I,L,M,F,P,S,T,W,Y;
X218A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X221A,R,D,C,Q,E,G,H,I,L,K,M,-
F,P,S,T,W,Y,V; X222A,R,N,D,C,E,G,H,I,L,K,M,F,P,S,W,Y,V;
X234A,D,C,G,H,I,K,M,F,P,S,T,W,Y,V; X235A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,-
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M,F,P,S,T,W,Y,V; X252A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V;
X253R,D,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y; X254A,R,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,-
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F,P,S,T,W,Y,V; X261A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
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X269A,N,C,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X271A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,-
T,W,Y,V; X272A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X275A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V; X276A,R,N,D,C,Q,E,G,H,I,L,M,F,P,-
S,T,W,Y,V; X278A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;

X280A,R,D,C,E,G,H,I,L,K,M,F,P,W,Y,V; X290W,Y; X291A,R,N,Q,G,H,I,L,M,F,P,S-
,T,W,Y,V; X293A,R,N,G,I,L,M,P,S,T,W,V; X294R,N,Q,G,H,I,L,M,F,P,S,T,W,Y;
X297A,G,H,I,L, M, F,P,W,Y,V; X298G,H,I,L,M,F,P,S,T,W,Y,V; X299A,G,H,M,P,S,T;
X300A,C,G,H,I,L,M,F,P,T,W,Y,V; X301 N,Q,H,I,L,M,F,P,S,T,W,Y,V; X302R,M,P,W,Y;
X303R,I,L,M,F,P,S,T,W,Y,V; X305G,I,L,M,F,P,S,T,W,Y,V;
X306Q,G,H,I,L,M,F,P,S,T,W,Y,V; X308A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X309N,Q,G,H,I,L,M,F,P,S,T,W,- Y,V; X310A,R,N,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y,V;
X314A,D,C,E,G,I,L,M,F,P,W,Y,- V; X315A,N,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V;
X316A,R,N,D,C,Q,E,G,H,I,L,K,M,F,- P,S,T,W,V;
X317R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X318A,R,N,D,C,Q,E,G,H,I,K,P,S,W,Y,V; X319A,R,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,-
W,Y,V; X328A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X332A,R,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X333A,N,D,C,G,I,M,F,P,S,T,Y,V;
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X336A,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V; X337A,R,N,C,Q,E,G,H,I,L,M,F,P,S,-
T,W,Y,V; X338A,R,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V; X340I,L,M,F,P,S,T,W,Y,V;
X341A,R,D,C,G,H,I,L,K,M,F,W,Y,V; X342R,I,L,M,F,P,S,T,W,Y,V;
X345R,N,D,C,Q,E,G,H,I,L,K,M,F,P,T,W,Y,V; X355A,R,N,D,C,Q,G,H,I,L,K,M,F,P,-
S,T,W,Y,V; X358A,R,D,C,G,K,M,P,W,Y; X363A,R,D,C,G,K,M,P,W,Y;
X370A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X371A,N,C,Q,E,G,H,I,L,K,M,F,-
P,S,T,W,Y,V; X373A,R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V;
X374A,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V; X375A,R,N,D,C,Q,G,H,I,L,K,M,F,P,-
S,T,W,V; X376A,RN,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V; X378R,N,D,C,Q,E,G,H,I,L-
,K,M,F,S,T,W,Y,V; X379A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,T,W,Y,V;
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X396A,R,D,C,G,K,M,P,W,Y; X398A,R,N,D,C,Q,E,G,H,I,L,M,F,W,Y,V;
X402R,C,G,K,M,P,W,Y; X403A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
X404R,I,L,M,F,P,S,T,W,Y,V; X405R,G,H,I,L,M,F,P,W,Y,V;
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P,S,T,W,Y,V; X414A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X415A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X416A,R,N,D,C,Q,E,G,H,I,L,K,-
M,F,P,S,T,W,Y,V; X417A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V;
X418A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X419A,R,N,D,C,Q,E,G,H,I,L,M,-
F,P,S,T,W,Y,V; X420A,N,D,C,E,G,H,I,L,K,M,F,S,T,W,Y,V;
X421A,R,N,D,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y,V; X422A,R,N,D,C,Q,E,G,H,I,L,K,M,-
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X432G,H,I,L,M,F,P,S,T,W,Y,V; X433A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
X434A,R,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X435Q,G,H,I,L,M,F,P,T,W,Y,V;
X436A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X437A,N,D,C,Q,E,G,H,i,L,K,M,-
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W,Y,V; X445A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X446A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X447A,N,D,C,G,H,I,L,M,F,P,S,-
T,W,Y,V; X448A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X450A,N,D,C,Q,E,G,I,L,K,M,F,P,S,T,W,V; X454A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,-
S,T,W,Y,V; X455A,R,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X456A,D,C,E,G,H,I,L,M,F,P,S,T,W,Y,V; X457A,R,N,D,C,Q,E,G,H,I,L,K,M,F,W,Y,- V;
X458A,R,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X459R,N,D,C,Q,E,G,H,I,L,K,M,-
F,S,W,Y,V; X460A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X461A,R,N,D,C,Q,E,G,H,I,L,M,F,P,S,W,Y,V; X463A,R,N,D,C,Q,E,H,I,L,K,M,F,P,-
S,T,W,Y,V; X464A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X465A,R,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X467A,R,N,D,C,Q,E,G,H,I,L,K,M,-
F,P,S,T,W,Y,V; X469A,R,N,D,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
X473N,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X474A,R,G,H,I,L,M,F,P,W,Y,V;
X475A,N,Q,G,H,I,L,M,F,P,S,T,W,Y,V; X476G,H,I,L,M,F,P,S,T,W,Y,V;
X482A,N,D,C,G,H,I,L,M,F,S,T,W,Y,V; X483A,N,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,- V
wherein (a) the variant has alpha-amylase activity and (b) each position

corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (Bacillus licheniformis alph-amylase).

Claims Text - CLTX (3):

3. A variant of a parent Termamyl-like alpha-amylase, comprising one or more of the following substitutions: X7A,R,N,D,C,Q,E,G,H,K,M,P,S,Y,V; X8C,M X9A,R,N,D,C,Q,G,H,M,P,S,T,W,Y,V; X11A,N,D,C,Q,G,H,I,L,M,P,S,T,W,Y,V- ; X12A,R,N,D,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X19A,R,N,D,C,Q,E,G,H,I,L,K,M,F-,P,S,T,W,Y,V; X21A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X22A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X25A,R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X32A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X40A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X41A,R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X46A,R,D,C,G,K,M,P,W,Y; X48R,N,D,C,Q,E,G,H,K,M,F,P,W,Y; X55A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X57R,N,D,C,Q,E,G,H,K,M,P,W; X58A,R,N,D,C,Q,E,G,H,K,M,S,T,W,Y; X60A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,- V; X77A,R,D,C,G,K,M,P,W,Y; X95A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X97A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X98A,R,D,C,G,K,M,P,W,Y; X99R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X100A,R,D,C,Q,E,G,H,I,K,M,F,P,S-,T,W,Y,V; X101A,N,C,Q,G,I,L,M,P,S,T,W,Y,V; X102N,D,C,Q,E,H,I,L,M,F,P,W,Y,V- ; X103A,N,D,C,Q,E,G,M,P,S,W,Y; X105A,N,C,Q,G,H,I,L,M,P,S,T,Y,V; X107R,N,D,Q,E,H,K,M,F,P,W,Y; X115R,N,D,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y,V; X118R,N,C,Q,E,H,I,L,K,M,F,P,S,T,W,Y,V; X135A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,-S,T,W,Y,V; X139A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X141A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X143A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,Y,V; X151A,R,N,D,C,Q,E,G,H,K,M,P,S,T,Y,V; X159A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,V; X160A,R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X161A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X162A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X163A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X166A,R,N,D,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V; X175A,R,D,C,G,K,M,P,W,Y; X177A,N,D,C,Q,E,H,I,L,K,M,P,S,T,W,Y,V; X183A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X186A,R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X187A,R,C,Q,E,G,H,I,L,K,M,F,P,W,Y,V; X192A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X199R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X200A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X202A,R,D,C,Q,E,.G,H,I,L,K,M,F,P,S,T,W,Y,V; X203A,R,D,C,G,K,M,P; X208A,R,N,D,C,Q,E,G,H,L,K,M,F,P,S,T,W,Y,V; X212A,N,C,Q,G,H,M,P,S,T,V; X215A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X219A,R,D,C,G,K,M,P,W,Y; X228A,R,N,D,C,Q,E,G,H,I,L,K,M,P,S,T,W,Y,V; X230A,R,N,D,C,Q,E,G,L,M,P,S,T,-W,Y,V; X233R,N,C,Q,E,G,H,I,K,M,P,S,T,W,Y; X236A,C,Q,G,H,I,M,P,S,T,V; X238A,R,N,D,C,Q,E,G,H,I,K,M,P,S,T,W,Y,V; X240A,R,N,D,C,Q,E,G,H,I,L,K,M,F,-P,S,T,W,Y,V; X241A,N,C,Q,G,H,P,S,T,V; X244A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,-T,W,Y,V; X248A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X256C,M; X258A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X259A,R,N,D,C,Q,E,G,H,K,M,P,-S,T,W,Y,V; X260R,N,D,C,Q,E,H,I,L,K,M,F,P,T,W,Y,V; X262A,R,D,C,G,K,M,P,W,Y; X270A,N,C,Q,G,I,L,M,F,P,S,T,W,Y,V; X273A,R,D,C,G,K,M,P,Y; X274A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X277A,R,N,D,C,Q,E,G,H,K,M,P,-S,W,Y,V; X281A,R,N,D,C,Q,E,G,K,M,P,S,T,W,Y,V; X283A,R,N,C,Q,E,G,I,L,K,M,F,-P,S,T,W,Y,V; X284A,R,N,D,C,Q,E,G,I,L,K,M,F,P,S,T,Y,V; X285A,R,D,C,Q,E,G,H,I,K,M,F,P,S,T,W,Y,V; X286A,R,D,C,Q,E,G,H,I,K,M,F,P,S,-T,W,Y,V;preferably X286N,C,Q,I,L,M,P,T,V,Y,F; X287R,N,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X288A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X289A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X292A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X295A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X296A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X304C,M; X307A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X312A,N,C,Q,G,H,I,L,M,F,P,S,-T,W,Y,V; X313A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X320R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X322R,N,D,C,Q,E,G,H,L,K,M,F,P,-S,T,W,Y,V; X323A,R,N,D,C,Q,E,G,I,L,K,M,F,P,S,T,W,Y,V;

X325A,R,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X326A,R,N,C,Q,E,G,M,P,S,T,W; X327A,R,C,G,H,I,L,K,M,P,S,T,W,Y,V; X329A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,T,W,-Y,V; X331N,D,C,Q,E,G,H,I,L,M,F,P,S,T,W,Y,V; X339A,R,N,C,Q,E,G,H,I,L,K,M,F,-P,S,T,W,Y,V; X343A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,Y,V; X344A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X346A,R,N,D,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X347A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X349R,N,D,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X350A,R,N,C,Q,G,H,I,L,K,M,F,P,S,-T,Y,V; X359R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X360N,D,Q,G,H,I,L,M,F,P,-S,T,W,Y,V; X369A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X377A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X380A,R,N,D,C,Q,E,G,H,K,P,S,-W,Y,V; X387A,R,N,D,C,Q,E,G,H,L,K,M,P,S,T,W,Y,V; X409N,C,Q,E,G,H,M,P,S,T,W,-Y,V; X410A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X411A,R,N,D,C,Q,E,G,H,I,-L,K,M,F,P,S,T,W,Y,V; X412R,N,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X423A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X424A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X426A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X427A,R,N,D,C,Q,E,G,H,K,M,P,S,T,Y,V; X428A,N,D,Q,E,G,H,I,M,F,P,S,W,Y,V; X429A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X430A,R,D,C,Q,E,G,H,I,L,K,M,-F,P,S,T,W,Y,V; X438C,M; X440R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X441A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X449A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X462A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X472A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y,V; X477A,R,N,D,C,Q,E,G,H,I,L,K,-M,F,P,S,T,W,Y,V; X479A,R,N,D,Q,E,G,H,I,L,K,M,F,P,S,W,Y,V; X480A,R,D,C,G,K,M,P,W,Y; X481A,R,N,D,C,Q,E,G,H,K,M,P,S,T,Y,V wherein (a) the variant has alpha-amylase activity and (b) each position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (Bacillus licheniformis alpha-amylase).

Claims Text - CLTX (4):

4. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions selected from the group of: A1 insertion; L3 insertion; N4 insertion; N17 insertion; D18 insertion; Q20 insertion; R23 insertion; R24 insertion; D28 insertion; Y56 insertion; L61 insertion or deletion; Y62 insertion; F67 insertion or deletion; H68 insertion; K80 insertion or deletion; G81 insertion or deletion; Q84 insertion; S85 insertion; H91 insertion or deletion; S92 insertion or deletion; K106 insertion or deletion; D110 insertion or deletion; D114 deletion; E119 insertion or deletion; D121 insertion; P122 insertion; A123 insertion; D124 insertion; R125 insertion; N126 insertion; R127 insertion; I129 insertion; G131 insertion; L134 insertion; K136 insertion; N172 insertion; E185 insertion; L196 insertion or deletion; P206 insertion or deletion; T217 insertion; W218 insertion; D231 insertion or deletion; A232 insertion or deletion; H235 insertion or deletion; N246 insertion; H247 insertion; R249 insertion; K251 insertion; F257 insertion or deletion; N278 insertion; G310 insertion or deletion; H316 insertion; P317 insertion; D328 insertion or deletion; G332 insertion or deletion; E355 insertion or deletion; Y358 insertion; Y363 insertion; Y367 insertion; K370 insertion; S373 insertion; R375 insertion; E376 insertion; K381 insertion; H382 insertion; R391 insertion or deletion; Y396 insertion; R413 insertion or deletion; E414 insertion or deletion; G415 insertion or deletion; D416 insertion; S417 insertion; S418 insertion; V419 insertion; A420 insertion; N421 insertion; S422 insertion or deletion; Y439 insertion; A445 insertion or deletion; G446 insertion or deletion; T448 insertion or deletion; H450 insertion; G454 insertion or deletion; N455 insertion; E458 insertion; P459 insertion; V460 insertion; V461 insertion; N463 insertion; S464 insertion; E465 insertion; W467 insertion; wherein (a) the alteration(s) are independently (as specified above): (i) an insertion of an amino acid downstream of the amino acid which occupies the position, or (ii) a deletion of the amino acid which occupies the position, (b) the variant has alpha-amylase activity and (c) each position

corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (Bacillus licheniformis alph-amylase).

Claims Text - CLTX (5):

5. A variant of a parent Termamyl-like alpha-amylase, comprising an alteration at one or more positions selected from the group of: L7 insertion or deletion; M8 insertion; Y10 insertion; F11 insertion; E12 insertion or deletion; M15 insertion; G19 insertion; H21 insertion; W22 insertion; L25 insertion; V40 insertion or deletion; W41 insertion; P43 insertion or deletion; P44 insertion or deletion; Y46 insertion; G55 insertion; Y59 insertion; Y77 insertion; G78 insertion or deletion; L90 insertion or deletion; I95 insertion; V97 insertion; Y98 insertion; G99 insertion; D100 insertion; V101 insertion; V102 insertion; H105 insertion or deletion; A109 insertion or deletion; V115 insertion or deletion; V118 insertion or deletion; I135 insertion; T139 insertion or deletion; F141 insertion or deletion; Y195 insertion; V208 insertion or deletion; W215 insertion; Y219 insertion; I236 insertion or deletion; F238 insertion or deletion; F240 insertion or deletion; W244 insertion; V248 insertion; M256 insertion; T258 insertion or deletion; V259 insertion or deletion; V312 insertion or deletion; V313 insertion or deletion; S320 insertion; T322 insertion or deletion; F323 insertion or deletion; D325 insertion or deletion; N326 insertion; H327 insertion or deletion; Q330 insertion or deletion; P331 insertion or deletion; Y348 insertion; A349 insertion or deletion; F350 insertion or deletion; P359 insertion or deletion; Q360 insertion; D365 insertion or deletion; M366 insertion; T369 insertion; I377 insertion; I384 insertion or deletion; L388 insertion or deletion; G423 insertion or deletion; L424 insertion or deletion; M438 insertion; G441 insertion or deletion; W449 insertion; I462 insertion; I479 insertion or deletion; Y480 insertion; V481 insertion or deletion; wherein (a) the alteration(s) are independently (as specified above): (i) an insertion of an amino acid downstream of the amino acid which occupies the position, or (ii) a deletion of the amino acid which occupies the position, (b) the variant has alpha-amylase activity and (c) each position corresponds to a position of the amino acid sequence of the parent Termamyl-like alpha-amylase having the amino acid sequence shown in SEQ ID NO: 8 (Bacillus licheniformis alph-amylase).

Claims Text - CLTX (6):

6. The variant of any of claims 1-5, wherein the parent Termamyl-like alpha-amylase is derived from a strain of B. licheniformis (SEQ ID NO: 8), B. amyloliquefaciens (SEQ ID NO: 10), B. stearothermophilus (SEQ ID NO: 6), Bacillus sp. (SEQ ID NO: 12 (M560), Bacillus sp. (SEQ ID NO: 2 (SP690)); Bacillus sp. (SEQ ID NO: 4 (SP722); Bacillus sp. #707 alpha-amylase (SEQ ID NO: 13); KSM-AP1378.

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TITLE: Alpha-amylase variant with altered properties

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INVENTOR-INFORMATION:

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PA	2000 01533	2000PA-2000 01533	October 13, 2000
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ABSTRACT:

The present invention relates to variants of parent alpha-amylases, which variant has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, specific activity, and altered pI, in particular higher pI.

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Summary of Invention Paragraph - BSTX (2):
[0001] The present invention relates to variants (mutants) of parent alpha-amylases, in particular of Bacillus origin, which variant has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, specific activity, and pI, in particular higher pI.

Detail Description Paragraph - DETX (2):
[0032] The object of the present invention is to provide an alpha-amylases,

in particular of Bacillus origin, which variants has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, specific activity, and altered pI, in particular higher pI.

Detail Description Paragraph - DETX (53):
[0077] Variants of the invention may have altered oxidation stability, in particular higher oxidation stability, in comparison to the parent alpha-amylase.

Detail Description Paragraph - DETX (77):
[0097] Important positions and mutations with respect to obtaining variants with improved stability at low pH are Aspargine substitutions. Preferred mutations include substitution or deletion of one or more Aspargine (Asn). Target Aspargines in SEQ ID NO: 2 (KSM-36) are N4, N17, N23, N34, N49, N68, N93, N96, N104, N121, N124, N147, N148, N161, N172, N179, N181, N183, N190, N192, N200, N278, N289, N291, N306, N326, N360, N371, N373, N393, N421, N430, N455, N463, N473, N482, which may be substituted with any other amino acid, or deleted, in particular N190F.

Detail Description Paragraph - DETX (93):
[0109] In relation to the above, a further aspect of the present invenbon relates to a method for generating a variant of a parent alpha-amylase, e.g. wherein the variant exhibits altered or increased thermal stability relative to the parent, the method comprising:

Detail Description Paragraph - DETX (96):
[0112] (c) screening for host cells expressing an alpha-amylase variant which has an altered property (e.g., pH-stability) relative to the parent alpha-amylase.

Detail Description Paragraph - DETX (117):
[0129] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing th transcription of the DNA sequence encoding an alpha-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis alpha-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens alpha-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral alpha-amylase, A. niger acid stable alpha-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (198):
[0186] Amylases: Suitable amylases (alpha and/or beta) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from Bacillus, e.g., a special strain of B. licheniformis, described in more detail in GB 1,296,839. Examples of useful alpha-amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the

variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

Detail Description Paragraph - DETX (253):

[0233] The assay can be used to screening of alpha-amylase variants having an improved stability at high pH compared to the parent enzyme and alpha-amylase variants having an improved stability at high pH and medium temperatures compared to the parent enzyme depending of the screening temperature setting.

Detail Description Paragraph - DETX (304):

[0270] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

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ABSTRACT:

The invention relates to a method of producing ethanol by fermentation, said method comprising a secondary liquefaction step in the presence of a thermostable acid alpha-amylase or, a thermostable maltogenic acid alpha-amylase.

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Detail Description Paragraph - DETX (100):
[0108] The "primary liquefaction" is preferably performed in the presence of an alpha-amylase, e.g., derived from a micro-organism or a plant. Preferred alpha-amylases are of fungal or bacterial origin. Bacillus alpha-amylases (often referred to as "Termamyl-like alpha-amylases"), variant and hybrids thereof, are specifically contemplated according to the invention. Well-known Termamyl-like alpha-amylases include alpha-amylase derived from a strain of B. licheniformis (commercially available as Termamyl.TM.), B. amyloliquefaciens, and B. stearothermophilus alpha-amylase. Other Termamyl-like alpha-amylases include alpha-amylase derived from a strain of the Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and

Biophysical Research Communications, 151 (1988), pp. 25-31. In the context of the present invention a Termamyl-like alpha-amylase is an alpha-amylase as defined in WO 99/19467 on page 3, line 18 to page 6, line 27. Contemplated variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467, and include the Bacillus stearothermophilus alpha-amylase (BSG alpha-amylase) variant, alpha-amylase TTC, having the following mutations delta(181-182)+N193F (also denoted I181*+G182*+N193F) compared to the wildtype amino acid sequence set forth in SEQ ID NO:3 disclosed in WO 99/19467. Contemplated alpha-amylase derived from a strain of Aspergillus includes Aspergillus oryzae and Aspergillus niger alpha-amylases.

Detail Description Paragraph - DETX (133):

[0141] Other contemplated Aspergillus glucoamylase variants include variants to enhance the thermal stability: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Furthermore, Clark Ford presented a paper on Oct. 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct 12-17, 1997, Abstract number: Abstract book p.0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an Aspergillus awamori glucoamylase to improve the thermal stability. Other glucoamylases include Talaromyces glucoamylases, in particular derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermopiles (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040082028 A1

TITLE: Pullulanase variants and methods for preparing such variants with predetermined properties

PUBLICATION-DATE: April 29, 2004

INVENTOR-INFORMATION:

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APPL-NO: 09/ 996024

DATE FILED: November 16, 2001

RELATED-US-APPL-DATA:

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COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00045	2000DK-PA 2000 00045	January 28, 2000

US-CL-CURRENT: 435/69.1, 435/18 , 435/210 , 435/325 , 435/6 , 702/19

ABSTRACT:

The present invention relates to pullulanase variants, wherein the variants have improved properties, for example, altered pH optimum, improved thermostabilty, altered substrate specificity, increased specific activity or altered cleavage pattern. The present invention also relates to methods of making pullulanase variants having at least one altered property based on the three-dimensional structure of a parent pullulanase.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of U.S. application Ser. No. 09/514,599 filed Feb. 28, 2000 and claims, under 35 U.S.C. 119, priority of Danish application no. PA 2000 00045 filed Jan. 12, 2000, the contents of which are fully incorporated herein by reference.

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Detail Description Paragraph - DETX (93):
[0123] Pullulanase Variants with Altered Stability

Detail Description Paragraph - DETX (94):
[0124] A variant with improved stability (typically increased thermostability) may be obtained by substitution with proline, substitution of

histidine with another amino acid, introduction of a disulfide bond, removal of a deamidation site, altering a hydrogen bond contact, filling in an internal structural cavity with one or more amino acids with bulkier side groups, introduction of interdomain interactions, altering charge distribution, helix capping, or introduction of a salt bridge.

Detail Description Paragraph - DETX (123):
[0153] Furthermore, it is envisaged from the structure that deletion of certain amino acid residues will confer increased stability, such as increased thermostability, to the thus produced variant. Variants, which are believed to be of particular importance, comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (125):
[0155] Other deletions which are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (127):
[0157] Furthermore, the following deletions are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detail Description Paragraph - DETX (130):
[0160] For example, it is envisaged that deletion of the below amino acid residues will confer increased stability, such as increased thermostability, to the thus produced variant of the pullulanase from Bacillus deramificans (SEQ ID NO: 3):

Detail Description Paragraph - DETX (132):
[0162] Other deletions which are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 3:

Detail Description Paragraph - DETX (134):
[0164] Furthermore, the following deletions are expected to confer increased stability, such as increased thermostability, to the pullulanase variant comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 3:

Detail Description Paragraph - DETX (149):
[0179] h) selecting a variant having increased stability and/or an altered temperature dependent activity profile as compared to the parent pullulanase.

Detail Description Paragraph - DETX (162):
[0192] A variant with improved stability (typically improved thermostability) as compared to the parent pullulanase may be obtained by introducing new interdomain and intradomain contacts, such as establishing inter- or intradomain disulfide bridges.

Detail Description Paragraph - DETX (169):
[0199] f) testing the stability of said variant; and

Detail Description Paragraph - DETX (171):
[0201] h) selecting a variant having increased stability as compared to the

parent pullulanase.

Detail Description Paragraph - DETX (179):
[0209] A variant with improved stability (typically improved thermostability) as compared to the parent pullulanase may be obtained by changing the surface charge distribution of the pullulanase. For example, when the pH is lowered to about 5 or below histidine residues typically become positively charged and, consequently, unfavorable electrostatic interactions on the protein surface may occur. By engineering the surface charge of the pullulanase one may avoid such unfavorable electrostatic interactions which in turn leads to a higher stability of the pullulanase.

Detail Description Paragraph - DETX (186):
[0216] f) testing the stability of said variant; and

Detail Description Paragraph - DETX (188):
[0218] h) selecting a variant having increased stability as compared to the parent pullulanase.

Detail Description Paragraph - DETX (208):
[0238] Variants with improved stability, in particular variants with improved thermostability, can be obtained by improving existing or introducing new interdomain or intradomain contacts. Such improved stability can be achieved by the modifications listed below.

Detail Description Paragraph - DETX (209):
[0239] Thus, one preferred embodiment of the invention relates to a variant of a parent pullulanase which has an improved stability and one or more salt bridges as compared to the parent pullulanase, wherein said variant comprises a modifications, e.g. a substitution, in a position corresponding to at least one of the following sets of positions in SEQ ID NO: 1:

Detail Description Paragraph - DETX (407):
[0437] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed

Detail Description Paragraph - DETX (420):
[0450] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a pullulanase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (436):
[0466] To screen for variants with increased stability, the filter with bound pullulanase variants can be pretreated prior to the detection step

described above to inactivate variants that do not have improved stability relative to the parent pullulanase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from pH 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection media.

Detail Description Paragraph - DETX (437):

[0467] To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from pH 2-12) at an elevated temperature (e.g., in the range from 50.degree.-110.degree. C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent pullulanase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Blue labeled pullulan and incubated until activity is detectable. As will be understood, thermostability and increased isoamylase activity may be tested simultaneously by using a detection plate containing immobilized Cibacron Red labeled amylopectin and incubate until activity is detectable. Moreover, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent pullulanase is inactivated, thereby allowing detection of only those variants with increased stability at the pH in question. To screen for variants with increased calcium-dependent stability, calcium chelators, such as ethylene glycol-bis(.beta.-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent pullulanase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040072718 A1

TITLE: Laundry detergent compositions comprising zwitterionic polyamines and mid-chain branched surfactants

PUBLICATION-DATE: April 15, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 679917

DATE FILED: October 6, 2003

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child 09980799 20011203 US

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non-provisional-of-provisional 60144113 19990716 US

non-provisional-of-provisional 60144111 19990716 US

US-CL-CURRENT: 510/499

ABSTRACT:

The present invention relates to laundry detergent compositions which provide enhance hydrophilic soil cleaning benefits, said compositions comprising:
a) from about 0.01% by weight of a zwitterionic polyamine;
b) from about 0.01% by weight, of a surfactant system comprising:
i) from 0% to 80% by weight, of a mid-chain branched alkyl sulfate surfactant;
ii) from 0% to 80% by weight, of a mid-chain branched aryl sulfonate

surfactant;
iii) optionally from 0.01% by weight, of a surfactant selected from the group consisting of anionic, nonionic, cationic, zwitterionic, ampholytic surfactants, and mixtures thereof;
c) the balance carriers and other adjunct ingredients.

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Detail Description Paragraph - DETX (136):
[0261] A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detail Description Paragraph - DETX (138):
[0263] A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase variant having a different proteolytic activity, stability, substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detail Description Paragraph - DETX (163):
[0287] Amylases suitable herein include, for example, .alpha.-amylases described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol.260, No. 1, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the Bacillus .alpha.-amylases, regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the aboveidentified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching,

as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a mutant in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B.licheniformis alpha-amylase, known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant alpha-Amylases" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate alpha-amylases but that improved oxidative stability amylases have been made by Genencor from B.licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific mutants, particularly important being M197L and M197T with the M197T variant being the most stable expressed variant. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase variants having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple mutant parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

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DOCUMENT-IDENTIFIER: US 20040063184 A1

TITLE: Fermentation processes and compositions

PUBLICATION-DATE: April 1, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 459143

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RELATED-US-APPL-DATA:

non-provisional-of-provisional 60413730 20020926 US

US-CL-CURRENT: 435/161, 435/105

ABSTRACT:

The present invention provides improved fermentation processes, including for use in an ethanol production process. The improved fermentation processes include applying esterases (such as, lipases, phospholipases and cutinases), laccases, phytases and/or proteases to a fermentation process. The improved fermentation process may also involve the addition of various growth stimulators for the fermenting microorganisms, including vitamins and mineral.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application No. 60/413,730 filed Sep. 26, 2002, the contents of which are fully incorporated herein by reference.

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Summary of Invention Paragraph - BSTX (62):
[0059] The liquefaction processes are typically carried out using an alpha-amylase. Preferred alphaamylases are of fungal or bacterial origin. More preferably, the alpha-amylase is a Bacillus alpha-amylases, such as, derived from a strain of B. licheniformis, B. amyloliquefaciens, and B. stearothermophilus. Other alpha-amylases include alpha-amylase derived from a strain of the Bacillus sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the alpha-amylase described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. Other alpha-amylase variants and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Other alpha-amylase include alpha-amylases derived from a strain of Aspergillus, such as, Aspergillus oryzae and Aspergillus niger alpha-amylases.

Summary of Invention Paragraph - BSTX (69):
[0066] Other Aspergillus glucoamylase variants include variants to enhance

the thermal stability, such as, G137A and G139A (Chen et al. (1996), Prot. Engng. 9,499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Other glucoamylases include Talaromyces glucoamylases, in particular, derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases contemplated include glucoamylases from the genus Clostridium, in particular C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).